Rhodamine-based chemosensor for Hg^{2+} in aqueous solution with a broad pH range and its application in live cell imaging†

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A new fluorescent probe, rhodamine B derivative (**1**) bearing an 8-hydroxyquinoline group, was synthesized and displayed highly selective and sensitive Hg^{2+}-amplified absorbance and fluorescence emission above 500 nm in aqueous solution with a broad pH range 4–9. It was found that mercury ions coordinate reversibly to **1** and the spirolactam ring of **1** was opened, forming a 1 : 1 metal–ligand complex. Furthermore, this sensor was applied for *in vivo* imaging in HeLa cells to confirm that **1** can be used as a fluorescent probe for monitoring Hg^{2+} in living cells.

Introduction

The design and construction of chemosensors with high selectivity and sensitivity for heavy metal cations has received considerable attention, as such metal ions can cause severe risks for human health and the environment.[1] Among them, mercury ion is considered as one of the most dangerous cations. The extreme toxicity of mercury and its derivatives results from its high affinity for thiol groups in proteins and enzymes, leading to the dysfunction of cells and consequently causing health problems.[2] The health concerns over exposure to mercury have motivated the exploration of efficient methods for the monitoring of mercury in biological and environmental samples. Although sophisticated analytical techniques, involving of atomic absorption, atomic emission and inductively coupled plasma spectroscopy, are currently used in the environment, there is a strong demand to develop inexpensive and real-time monitoring methods for the detection of Hg^{2+} and other heavy metals.

Chemosensors based on the metal ion induced changes in fluorescence appear to be particularly attractive due to the simplicity and high detection limit of the fluorescence.[3] To date, considerable efforts have been devoted to the development of selective and efficient fluorescent chemosensors for the detection of mercury in biological and environmental samples.[4]

Rhodamine spirolactam based chemosensors are especially attractive due to their excellent spectroscopic properties of large molar extinction coefficient (ε), high emission quantum yields (Φ) and long absorption and emission wavelength elongated to visible region.[5] These sensors are nonfluorescent and colorless, whereas ring-opening of the corresponding spirolactam induced by metal ions gives rise to strong fluorescence emission and a pink color. Inspired by this strategy, many intelligent systems using rhodamine derivatives have been developed as fluorescent chemosensors for Hg^{2+}.[6,7] In these systems, the signal was transduced either through opening the spirolactam ring upon mercury coordination[6] or through chemodosimetric reactions of the thiocarbonyl activated by the strong affinity of mercury for sulfur.[7] However, the former usually have cross-sensitivities toward other metal cations or delayed responses; the latter suffer from the biggest problem, that is, they react with mercury irreversibly and cannot be reused for further analysis. Therefore, the search for new rhodamine sensors with the characteristics of high selectivity and affinity, sensitive and reversible turn-on response to Hg^{2+}, as well as good water-solubility, cell-permeability, and broad pH range has been the focus of extensive investigation.

Herein, we introduced a rhodamine B derivative (**1**) bearing a 8-hydroxyquinoline group as a reversible, selective and sensitive fluorescent and colorimetric sensor for Hg^{2+} at a board pH range 4–9 (Fig. 1). In our condition, "Off-on" type fluorescent and colorimetric changes were observed only for Hg^{2+}. Furthermore, this sensor could be applied for *in vivo* imaging in HeLa cells to confirm that our fluoroprobe can detect Hg^{2+} in living cells.

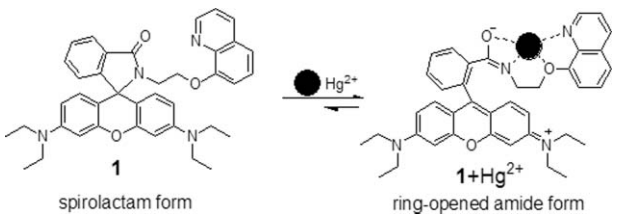

Fig. 1 Chemical structures of **1** and **1**-Hg^{2+}.

Results and discussion

The rhodamine derivative **1** was prepared from Rhodamine B through a three-step procedure [(1) $NH_2CH_2CH_2OH$, EtOH, 95%; (2) MsCl, Et_3N, CH_2Cl_2; (3) K_2CO_3, acetone and 8-hydroxyquinoline, 71.4%]. The structure of **1** was confirmed by ^1H NMR, ^{13}C NMR, MS (ESI†) and X-ray analysis.[8] A single crystal of **1** was grown from a MeOH–ethyl acetate solution and was characterized using X-ray crystallography (Fig. 2). The crystal structure clearly represents the unique spirolactam-ring formation. Two planes of the spiro of the rhodamine framework are coordinated in a mutually vertical position. The contents of the unit cell also includes an ethyl acetate molecule.

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† Electronic supplementary information (ESI) available: ^1H NMR, ^{13}C NMR and ESI-MS of **1**; IR spectra of **1** and **1**–Hg^{2+} complex in KBr disks; Time-dependent change in fluorescence intensity of **1** after Hg^{2+} addition; Determination of binding constant of the complex; Selectivity investigation by absorption spectra. CCDC reference numbers 773181. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c0ob00013b

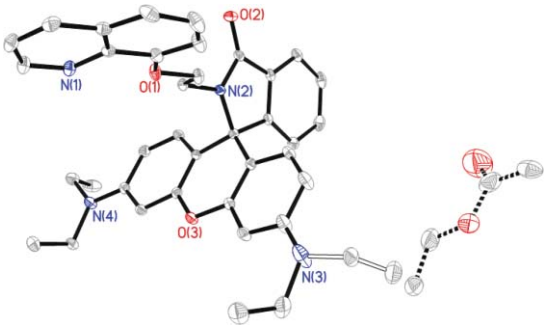


Fig. 2 View of the structure of **1** with displacement atomic ellipsoids drawn at the 30% probability level. All hydrogen atoms are omitted for clarity.

1 was designed to chelate with metal ions *via* its 8-hydroxyquinoline N and O,[6e,9] and ring-opened amide N and O (Fig. 1). The spirolactam moiety of **1** acted as a signal switcher, which was envisioned to turn on when the mercury ion was bound. An acetonitrile–water solution of **1** is colorless and nonfluorescent, indicating that the spirolactam form of **1** exists predominantly. The characteristic peak of the 9-carbon of **1** near 64 ppm in the ^{13}C NMR also supports this consideration.[10] As we expected, addition of Hg^{2+} to a MeCN–water solution of **1** caused an obvious pink color and bright orange fluorescence rapidly as a result of the Hg^{2+}-induced ring opening of the spirolactam form, and other ions of our interest, such as Na^+, K^+, Mg^{2+}, Mn^{2+}, Fe^{2+} , Ca^{2+}, Zn^{2+}, Co^{2+}, Pb^{2+}, Cu^{2+}, Cd^{2+}, Ni^{2+} and Ag^+, showed little interference (Fig. 3). IR spectra of **1** and **1**-Hg^{2+} were taken in KBr disks, respectively (Fig. S1, ESI†). The peak at 1697.2 cm^{-1}, which corresponds to the characteristic amide carbonyl absorption of **1**, was shifted to 1689 cm^{-1} upon chelating with Hg^{2+}, indicating that rhodamine carbonyl group is involved in Hg^{2+} coordination. The ^1H NMR-titration experiments showed that H atoms besides quinoline N atom displayed an apparent downshift upon addition of Hg^{2+}, indicating that 8-hydroxyquinoline group is also involved in Hg^{2+} coordination (Fig. S2, ESI†). The above results suggested that **1** can serve as a selective "naked-eye" and fluorescent sensor for Hg^{2+}.

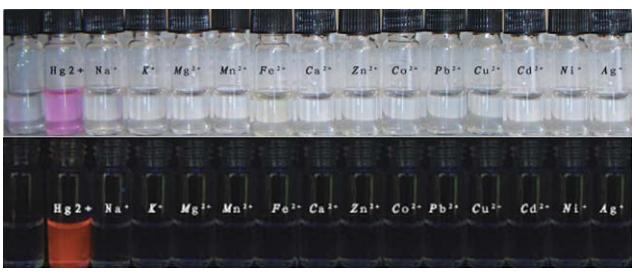


Fig. 3 The fluorescent (bottom) and the color (top) changes of **1** to various metal ions in MeCN–water solution (95/5, v/v, pH 7.2). [**1**] = 20 μM, [Hg^{2+}] = 50 μM and the concentration of other metal ions was 100 μM.

To apply **1** in more complicated systems, like in organism and environment, the spectra responses of **1** in the absence and presence of Hg^{2+} in different pH values were evaluated. Fig. 4 shows the spectra responses of **1** obtained without and with Hg^{2+} as a function of pH. Without Hg^{2+}, no obvious characteristic

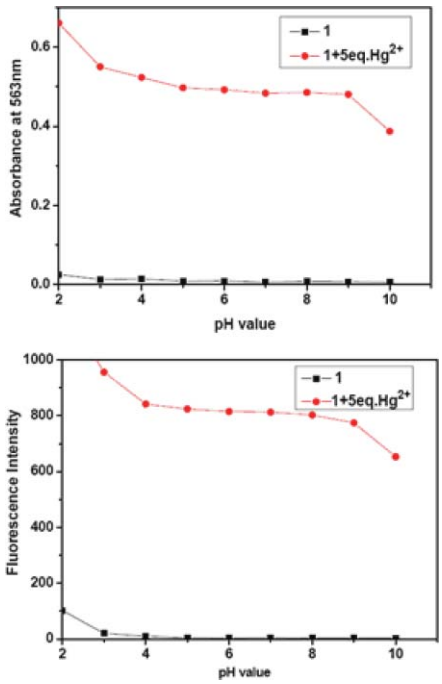


Fig. 4 Changes in absorption (563 nm) and fluorescence intensity (λ_{ex} = 530 nm, λ_{em} = 586 nm) of **1** (20 μM) in a MeCN–water mixture (95/5, v/v) measured with and without Hg^{2+} (5 equiv.) as a function of pH.

absorption or fluorescence of rhodamine could be observed for **1** between pH 4.0 and 9.0. Upon addition of Hg^{2+}, **1** responds stably to Hg^{2+} in the same region without any interference by protons. When the pH value is lower than 4.0, the spectra response also occurs upon the coordination of Hg^{2+}, but the intensity of the free **1** increases slowly with the decreasing pH values. These results indicate that **1** successfully reacts with Hg^{2+} and allows Hg^{2+} detection in a wide pH range.

The spectra characteristics of **1**–Hg^{2+} were found to be strongly dependent on the water content (Fig. S3, ESI†). With the increase of H_2O content, the fluorescence intensity of the solution is decreased. Here, to get a better sensitivity, a MeCN–water solution (95/5, v/v, pH 7.2) was selected as a testing system to investigate the chemical response of **1** to Hg^{2+} at room temperature. A time course study revealed the recognizing event could complete in less than 1 min (Fig. S4, ESI†). This feature of real-time response to Hg^{2+} is particularly important in practical application. The UV/visible spectrum of **1** (20 μM) shows no any absorbance above 500 nm, which is ascribed to its spirolactam form in solution. Upon addition of Hg^{2+} to a solution of **1**, the solution turned from colorless to pink, and the absorbance was significantly enhanced with a new peak appearing at around 560 nm (Fig. 5a), clearly suggesting the formation of the ring-opened amide form of **1** as a result of Hg^{2+} binding. Moreover, a clear isosbestic point at 330 nm in the absorption spectra indicates that the binding of **1** with Hg^{2+} produces a single component. The association constant for Hg^{2+} was estimated to be 2.18×10^6 M^{-1} (R^2 = 0.9916) on the basis of nonlinear fitting of the titration curve assuming 1 : 1 stoichiometry (Fig. S5, ESI†). Also, this 1 : 1 binding mode was supported by a Job plot (Fig. 5a inset).

The complexation of Hg^{2+} by **1** was also investigated by means of fluorescence titration in MeCN–water solution (95/5, v/v,

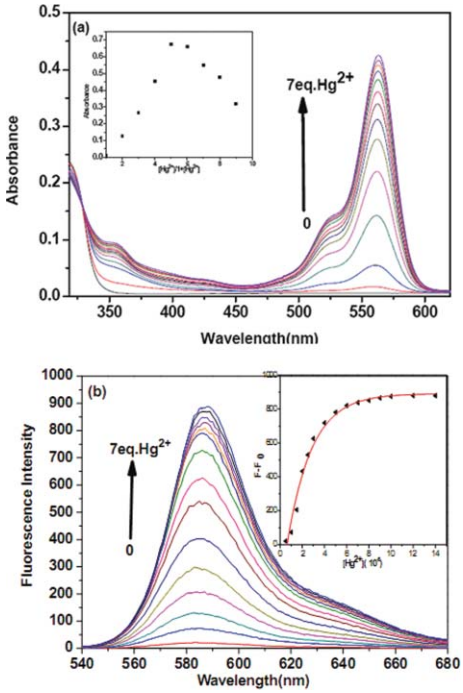


Fig. 5 Changes in absorption (a) and fluorescence emission (b) spectra of **1** (20 μM) in MeCN–water solution (95/5, v/v, pH 7.2) with various amounts of Hg^{2+} ions (λ_{ex} = 530 nm). Each spectrum was acquired 1 min after Hg^{2+} addition. Inset (a): Job's plots of the complexation between **1** and Hg^{2+}. Total concentration of **1** + Hg^{2+} was kept constant at 100 μM. Inset (b): Fluorescence titration profile (at 586 nm) *vs.* concentration of Hg^{2+} in solution for **1**.

pH 7.2). Upon addition of Hg^{2+}, a new emission band of **1** showing a maximum at 586 nm appeared (Fig. 5b). In the presence of 7 equiv. of Hg^{2+}, the mixture showed an intense red fluorescence, and $a > 1200$-fold enhancement in the fluorescence intensity at 586 nm was estimated. Fluorescence titration profile at 586 nm *versus* concentration of Hg^{2+} is shown in Fig. 5b inset. Based on nonlinear fitting of the titration curve assuming 1 : 1 stoichiometry, the association constant for Hg^{2+} was estimated to be 1.27×10^6 M^{-1} (R^2 = 0.9898) (Fig. S6, ESI†), which is comparable to that of absorbance. The high enhancement factor (>1200) of fluorescence of **1** upon the binding of Hg^{2+} and the large association constant ($K_a > 10^6$) of the **1**–Hg^{2+} complex suggested the possibility of Hg^{2+} sensing at a very low concentration level by **1**. When **1** was employed at 1 μM in a MeCN–water solution (95/5, v/v, pH 7.2), the fluorescent intensity of **1** was proportional to the concentration of Hg^{2+} added (Fig. 6). The detection limit was measured to be 1.14 ppb at S/N = 3, which is lower than the U.S. Environmental Protection Agency's limit (2 ppb) for drinking water.[11]

An important feature of **1** is its high selectivity toward the Hg^{2+} over the other competitive species. Changes of fluorescence and UV/Vis spectra of **1** (20 μM) caused by Hg^{2+} (100 μM) and miscellaneous cations (100 μM) including Na^+, K^+, Mg^{2+}, Mn^{2+}, Fe^{2+}, Ca^{2+}, Zn^{2+}, Co^{2+}, Pb^{2+}, Cu^{2+}, Cd^{2+}, Ni^{2+} and Ag^+ in a MeCN–water solution (95/5, v/v, pH 7.2) are recorded in Fig. 7 and Fig. S7 (ESI†), respectively. The miscellaneous competitive cations did not lead to any significant absorption in the visible region and fluorescence changes (Fig. 7a). Moreover, in the presence of miscellaneous competitive cations, the Hg^{2+} ion still resulted in the

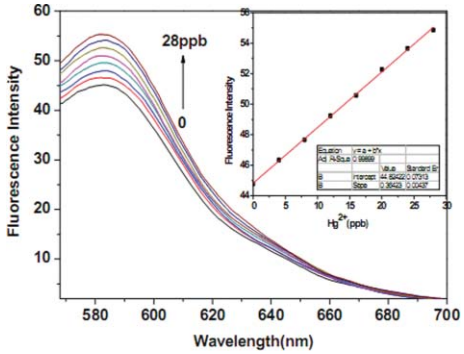


Fig. 6 Fluorescence response of **1** (1 μM) to Hg^{2+} in a MeCN–water solution (95/5, v/v, pH 7.2). The excitation wavelength was 530 nm, and the splits were 10 nm. Inset: titration curve of $I_{586\,nm}$ *vs.* Hg^{2+} concentration.

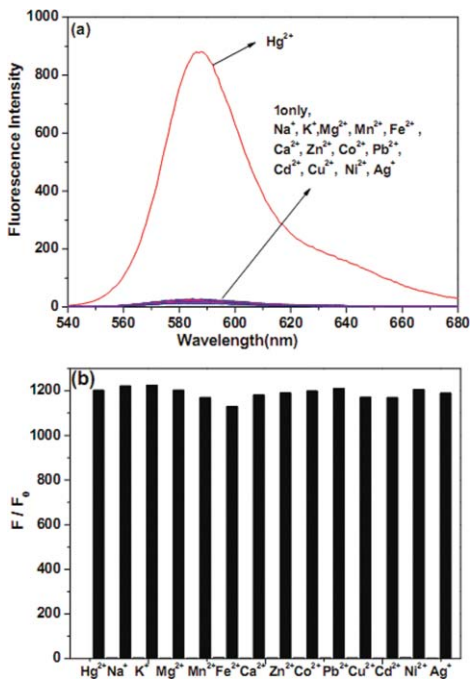


Fig. 7 (a) The fluorescence spectra of **1** (20 μM) upon addition of 100 μM of Hg^{2+} and various other metal ions in a MeCN–water solution (95/5, v/v, pH 7.2). (b) Fluorescence response of **1** (20 μM) to 100 μM of Hg^{2+} in a MeCN–water solution (95/5, v/v, pH 7.2) containing 100 μM of various metal ions. λ_{ex} = 530 nm, λ_{em} = 586 nm.

similar absorption and fluorescence changes (Fig. 7b). The results confirmed the remarkable selectivity of the probe **1** for Hg^{2+}.

For a chemical sensor to be widely employed in the detection of specific analyses, the reversibility is an important aspect. In light of strong binding ability of the iodide anion toward Hg^{2+}, the reversibility of the system was investigated by introduction of iodide anion (Fig. 8 and Fig. S8, ESI†). Upon addition of iodide anion, the color of the mixture of **1** and Hg^{2+} changed from pink to colorless, and fluorescent emission intensity of the system was quenched, indicating that iodide anion replaced the receptor **1** to coordinate Hg^{2+}.[6e,6i] Thus, reversible response toward Hg^{2+} implies that **1** is a chemosensor not a chemodosimeter of Hg^{2+}.

Taking into account the advantages of real-time response, extremely high sensitivity and selectivity of **1** for Hg^{2+}, **1** was used for *in vitro* Hg^{2+} detection in living cells. After HeLa cells

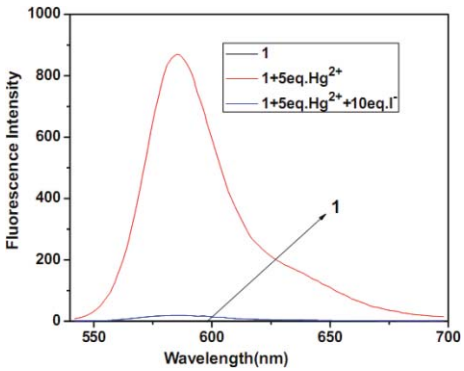


Fig. 8 The emission spectra of **1** (20 μM, MeCN–water solution, 95 : 5, v/v, pH=7.2) in the presence of 5 equiv. of Hg^{2+} and 10 equiv. of KI.

were incubated with 20 μM of **1** for 30 min at 37 °C, and then treated with different concentrations of Hg^{2+} from 0, 20, 50 μM, their fluorescence images were taken by fluorescence microscopy. As shown in Fig. 9, these fluorescence images display Hg^{2+} concentration dependence: the stronger fluorescence images of HeLa cells are those treated with the higher concentrations of Hg^{2+}. Under the same experimental conditions, no fluorescence was imaged in HeLa cells incubated with **1** only. These results demonstrate that **1** might be used for detecting Hg^{2+} in biological samples.

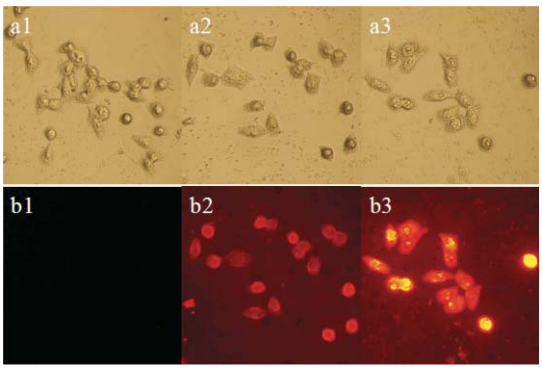


Fig. 9 Fluorescence images of Hg^{2+} in HeLa cells with 20 μM solution of **1** for 30 min at 25 °C. Bright-field transmission image (a1–a3) and fluorescence image (b1–b3) of HeLa cells incubated with 0 μM, 20 μM and 50 μM of Hg^{2+} for 30 min, respectively.

Conclusions

In summary, we have synthesized a new rhodamine-based chemosensor **1** linked with 8-hydroxyquinoline, which displayed a reversible absorption and fluorescence enhancement response to Hg^{2+} *via* a 1 : 1 binding mode in a board pH range. Its selectivity toward Hg^{2+} is very high because little interference was observed for other commonly coexistent metal ions. The sensitivity of **1** for Hg^{2+} can be lower than 2 ppb in MeCN–water solution (95 : 5, v/v, pH=7.2) by the fluorescence spectra method. Furthermore, the sensor was applied for *in vivo* imaging of Hg^{2+} using HeLa cells.

Experimental section

Materials and methods

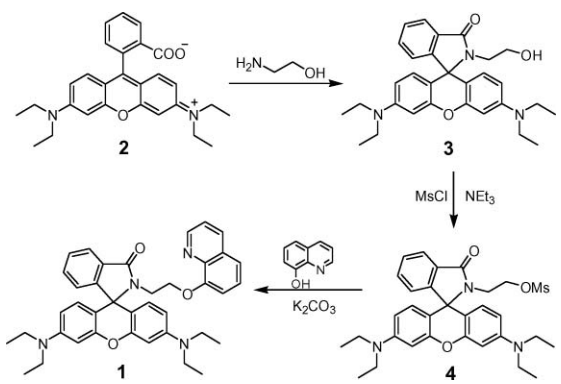
Commercially available compounds were used without further purification. Solvents were dried according to standard procedures. All reactions were magnetically stirred and monitored by thin-layer chromatography (TLC). Flash chromatography (FC) was performed using silica gel 60 (230–400 mesh). Deionized water was used throughout all experiments. Solutions of Fe^{2+} and Ca^{2+} were prepared from their chloride salts; solutions of Hg^{2+}, Na^{+}, K^{+}, Mg^{2+}, Mn^{2+}, Fe^{2+}, Ca^{2+}, Zn^{2+}, Co^{2+}, Pb^{2+}, Cu^{2+}, Cd^{2+}, Ni^{2+} and Ag^{+} were prepared from their nitrate salts. Absorption spectra were taken on an Agilent 8453 spectrophotometer. Fluorescence spectra were taken on Varian Cary Eclipse fluorescence spectrometer. The ^{1}H NMR and ^{13}C NMR spectra were recorded at 300 and 75 MHz, respectively. The following abbreviations were used to explain the multiplicities: *s* = singlet; *d* = doublet; *t* = triplet; *q* = quartet; *m* = multiplet; br = broad. Mass spectra were obtained using electron ionization (EI) mass spectrometer.

Cell Culture

The HeLa cell line was provided by Key Laboratory of Chemical Biology and Molecular Engineering of Ministry of Education (China). Cells were grown in RMPI 1640 medium supplemented with 10% FBS (Fetal Bovine Serum) and 1% antibiotics at 37 °C in humidified environment of 5% CO_2. Cells were plated on 6-well plate at 5 × 106 cells per well and allowed to adhere for 12 h. Fluorescence imaging was performed with an Olympus fluorescence microscope (BX51, Olympus, Japan). Before the experiments, cells were washed with physiological saline and then incubated with **1** (20 μM) in Ethanol-physiological saline (1 : 99, v/v) for 30 min at 37 °C. Experiments to assess Hg^{2+} uptake were performed in the same media supplemented with 0, 20, 50 μM $Hg(NO_3)_2$ for 30 min at 37 °C. Cell imaging was then carried out after washing cells with physiological saline.

Synthesis of probe 1 (Scheme 1)

The compound **3** was synthesized from the literature methods.[12] In a 100 mL round-bottomed flask were placed **3** (1.00 g, 2.06 mmol), triethylamine (3.15 g, 3.10 mmol) and dichloromethane (50 mL) under argon atmosphere. The mixture was cooled in an ice bath and then 190 μL of methanesulfonyl chloride was added



Scheme 1 Synthetic route of **1**.

dropwise. The reaction mixture was stirred at room temperature for overnight. It was washed successively with water (2 × 30 mL) and brine (30 mL). The organic layer was dried over Na_2SO_4 and evaporated under vacuo. The residue (**4**) obtained was directly used for next step without further purification.

To the above mesylate (**4**) was added 20 mL of dry acetone, 0.30 g (2.06 mmol) 8-hydroxyquinoline and anhydrous potassium carbonate (1.142 g, 8.27mmol) under inert atmosphere, and the reaction mixture was refluxed for 12 h. Then it was allowed to cool at room temperature and then filtration. The filtrate was evaporated under vacuo. The crude product was purified by flash column chromatograph using petroleum ether/ethyl acetate (1 : 1.7, v/v) as an eluent. The desired product (**1**) was obtained as light yellow solid in 71.4% yield (0.9 g). mp: 119–120 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 1.17 (12H, t, J = 6.9 Hz), 3.33 (8H, q), 3.69 (2H, t, J = 8.7 Hz), 4.13 (2H, t, J = 7.5 Hz), 6.28 (2H, q), 6.37 (2H, d), 6.49 (2H, d), 7.14 (1H, d), 7.35 (3H, m), 7.48 (3H, m), 7.94 (1H, d), 8.07 (1H, d), 8.84 (1H, d). ^{13}C NMR (CDCl3, 75 MHz) δ (ppm): 14.8, 38.4, 44.9, 65.5, 78.1, 98.4, 105.4, 108.6, 109.3, 122.0, 123.2, 124.5, 127.6, 129.9, 133.2, 136.4, 140.6, 149.7, 154.2, 168.9. IR (KBr pellet, cm^{-1}): 3076, 2966, 2929, 1737, 1697, 1631, 1614, 1517, 1222, 1151, 1070, 1012, 823, 794, 757, 462. HRMS [ESI]: m/z, calcd for [(M+H)] 613.3173; Found 613.3173; calcd for [(M+Na)]$^+$ 635.2993, Found 635.2988.

Acknowledgements

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Notes and references

1 (*a*) J. P. Desvergne, A. W. Czarnik, *Chemosensors of Ion and Molecule Recognition*Kluwer: Dordrecht, 1997; (*b*) *Fluorescent Chemosensors for Ion and Molecule Recognition*, A. W. Czarnik, Ed.; American Chemical Society, Washington, DC, 1992; (*c*) A. P. de Silva, H. Q. N. Gunaratne, T. Gunnlaugsson, A. J. M. Huxley, C. P. McCoy, J. T. Rademacher and T. E. Rice, *Chem. Rev.*, 1997, **97**, 1515.

2 (*a*) P. Grandjean, P. Weihe, R. F. White and F. Debes, *Environ. Res.*, 1998, **77**, 165; (*b*) T. Takeuchi, N. Morikawa, H. Matsumoto and Y. Shiraishi, *Acta Neuropathol.*, 1962, **2**, 40; (*c*) M. Harada, *Crit. Rev. Toxicol.*, 1995, **25**, 1.

3 (*a*) J. F. Callan, A. P. de Silva and D. C. Magri, *Tetrahedron*, 2005, **61**, 8551; (*b*) R. Martínez-Máñez and F. Sancanón, *Chem. Rev.*, 2003, **103**, 4419; (*c*) A. W. Czarnik, *Acc. Chem. Res.*, 1994, **27**, 302; (*d*) J. S. Kim and D. T. Quang, *Chem. Rev.*, 2007, **107**, 3780.

4 (*a*) H. Li, Y. Li, Y.-Q. Dang, L.-J. Ma, Y. Wu, G. Hou and L. Wu, *Chem. Commun.*, 2009, 4453; (*b*) A. Caballero, R. Martinez, V. Lloveras, I. Ratera, J. Vidal-Gancedo, K. Wurst, A. Tárraga, P. Molina and J. Veciana, *J. Am. Chem. Soc.*, 2005, **127**, 15666; (*c*) A. Dhir, V. Bhalla and M. Kumar, *Org. Lett.*, 2008, **10**, 4891; (*d*) Y. Shiraishi, S. Sumiya and T. Hirai, *Org. Biomol. Chem.*, 2010, **8**, 1310; (*e*) G. Zhang, D. Zhang, S. Yin, X. Yang, Z. Shuai and D. Zhu, *Chem. Commun.*, 2005, 2161; (*f*) M.-L. Ho, K.-Y. Chen, G.-H. Lee, Y.-C. Chen, C.-C. Wang, J.-F. Lee, W.-C. Chung and P.-T. Chou, *Inorg. Chem.*, 2009, **48**, 10304; (*g*) M. H. Lee, J.-S. Wu, J. W. Lee, J. H. Jung and J. S. Kim, *Org. Lett.*, 2007, **9**, 2501; (*h*) J. H. Kim, H. J. Kim, S. H. Kim, J. H. Lee, J. H. Do, H.-J. Kim, J. H. Lee and J. S. Kim, *Tetrahedron Lett.*, 2009, **50**, 5958; (*i*) Y. Shiraishi, H. Maehara, K. Ishizumi and T. Hirai, *Org. Lett.*, 2007, **9**, 3125; (*j*) M. G. Choi, Y. H. Kim, J. E. Namgoong and S.-K. Chang, *Chem. Commun.*, 2009, 3560; (*k*) X. Chen, S.-W. Nam, M. J. Jou, Y. Kim, S.-J. Kim, S. Park and J. Yoon, *Org. Lett.*, 2008, **10**, 5235; (*l*) K. C. Song, J. S. Kim, S. M. Park, K.-C. Chung, S. Ahn and S.-K. Chang, *Org. Lett.*, 2006, **8**, 3413; (*m*) Y. Shiraishi, S. Sumiya, Y. Kohno and T. Hirai, *J. Org. Chem.*, 2008, **73**, 8571; (*n*) H. Lu, L. Xiong, H. Liu, M. Yu, Z. Shen, F. Li and X. You, *Org. Biomol. Chem.*, 2009, **7**, 2554; (*o*) M. Yuan, Y. Li, J. Li, C. Li, X. Liu, J. Lv, J. Xu, H Liu, S. Wang and D. Zhu, *Org. Lett.*, 2007, **9**, 2313; (*p*) M.-H. Ha-Thi, M. Penhoat, V. Michelet and I. Leray, *Org. Lett.*, 2007, **9**, 1133.

5 J. R. Lakowicz, *Principles of Fluorescence Spectroscopy*, 3rd Ed.; Springer: New York, 2006; p 67.

6 (*a*) M. Suresh, S. Mishra, S. K. Mishra, E. Suresh, A. K. Mandal, A. Shrivastav and A. Das, *Org. Lett.*, 2009, **11**, 2740; (*b*) X. Zhang, Y. Shiraishi and T. Hirai, *Tetrahedron Lett.*, 2008, **49**, 4178; (*c*) D. Wu, W. Huang, C. Duan, Z. Lin and Q. Meng, *Inorg. Chem.*, 2007, **46**, 1538; (*d*) H. Zheng, Z.-H. Qian, L. Xu, F.-F. Yuan, L.-D. Lan and J.-G. Xu, *Org. Lett.*, 2006, **8**, 859; (*e*) H. Yang, Z. Zhou, K. Huang, M. Yu, F. Li, T. Yi and C. Huang, *Org. Lett.*, 2007, **9**, 4729; (*f*) J. Huang, Y. Xu and X. Qian, *J. Org. Chem.*, 2009, **74**, 2167; (*g*) M. H. Lee, J.-S. Wu, J. W. Lee, J. H. Jung and J. S. Kim, *Org. Lett.*, 2007, **9**, 2501; (*h*) Y. Shiraishi, S. Sumiya, Y. Kohno and T. Hirai, *J. Org. Chem.*, 2008, **73**, 8571; (*i*) D. Wu, W. Huang, Z. Lin, C. Duan, C. He, S. Wu and D. Wang, *Inorg. Chem.*, 2008, **47**, 7190; (*j*) A. Jana, J. S. Kim, H. S. Jung and P. K. Bharadwaj, *Chem. Commun.*, 2009, 4417.

7 (*a*) W. Liu, L. Xu, H. Zhang, J. You, X. Zhang, R. Sheng, H. Li, S. Wu and P. Wang, *Org. Biomol. Chem.*, 2009, **7**, 660; (*b*) W. Shi and H. Ma, *Chem. Commun.*, 2008, 1856; (*c*) Y.-K. Yang, K.-J. Yook and J. Tae, *J. Am. Chem. Soc.*, 2005, **127**, 16760; (*d*) X. Zhang, Y. Xiao and X. Qian, *Angew. Chem., Int. Ed.*, 2008, **47**, 8025; (*e*) J.-S. Wu, I.-C. Hwang, K. S. Kim and J. S. Kim, *Org. Lett.*, 2007, **9**, 907.

8 The crystal data of **1**·(EtOAc): molecular formula: $C_{41}H_{44}N_4O_4$, crystal size: 0.26 × 0.22 × 0.18, M = 656.80, Triclinic, space group: $P\bar{1}$, a = 12.014(7) Å, b = 12.413(5) Å, c = 13.062(7) Å, α = 70.46(3)°, β = 85.28(3)°, γ = 73.53(2)°, V = 1760.3(15), T = 113(2) K, d_c = 1.239 g cm^{-3}, $F(000)$ = 700, 13376 reflections measured, 6196 unique (R_{int} = 0.0527). The final R_1 = 0.0516 ($I > 2\sigma$), 0.0733 (all data), wR_2 = 0.1237 ($I > 2\sigma$), 0.1324 (all data).(.

9 (*a*) J.-S. Youk, Y. H. Kim, E.-J. Kim, N. J. Youn and S.-K. Chang, *Bull. Korean Chem. Soc.*, 2004, **25**, 869; (*b*) S. Y. Moon, N. R. Cha, Y. H. Kim and S.-K. Chang, *J. Org. Chem.*, 2004, **69**, 181; (*c*) K. S. Song, J. S. Kim, S. M. Park, K.-C. Chung, S. Ahn and S.-K. Chang, *Org. Lett.*, 2006, **8**, 3413; (*d*) H. Zhang, L.-F. Han, K. A. Zachariasse and Y.-B. Jiang, *Org. Lett.*, 2005, **7**, 4217; (*e*) K. G. Vaswani and M. D. Keränen, *Inorg. Chem.*, 2009, **48**, 5797.

10 U. Anthoni, C. Christophersen, P. Nielsen, A. Puschl and K. Schaumburg, *Struct. Chem.*, 1995, **6**, 161.

11 Mercury Update: Impact of Fish Advisories. EPA Fact Sheet EPA-823-F-01-011; EPA, Office of Water: Washington, DC, 2001.

12 A. Chatterjee, M. Santra, N. Won, S. Kim, J. K. Kim, S. B. Kim and K. H. Ahn, *J. Am. Chem. Soc.*, 2009, **131**, 2040.